One-Step Process for Desizing and Bleaching of Cotton Fabrics Using the Combination of Amylase and Glucose Oxidase Enzymes

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ABSTRACT: Bacterial α amylases, widely used in the desizing of the gray cotton fabrics, convert the starch present in the warp yarns into glucose, a reducing sugar. An attempt has been made to convert the glucose released by amylases in the desizing process into hydrogen peroxide using glucose oxidase enzymes and use the hydrogen peroxide for bleaching of cotton fabrics, in a single step. Conversion of glucose, into hydrogen peroxide, is influenced by aeration of the reaction bath and concentration of the glucose oxidase. Significant improvement in the whiteness and absorbency, reduction in the extractable impurities and clear surface morphology were also observed in the samples obtained from the one-step process. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 123: 2445–2450, 2012

Key words: absorbency; amylase; glucose oxidase; hydrogen peroxide; whiteness index

INTRODUCTION

α amylases cleave the starch into various oligomers, finally to glucose as the end product in the hydrolysis and desizing of the gray cotton fabrics using amylases has been successfully implemented in the commercial processes.[1–7] Glucose oxidase catalyzed reaction on glucose, in presence of oxygen, produces hydrogen peroxide, and shows the potential for bleaching of cotton fabrics. Glucose is oxidized to δ galactone by the cofactor of glucose oxidase (flavin adenine dinucleotide, FAD), which in turn reduces to its hydride form ($FADH_2$). Subsequently, oxygen present in the reaction system is reduced to hydrogen peroxide while $FADH_2$ is reoxidized to FAD.[8,9] Attempts have been made to utilize the spent-desize bath for bleaching using immobilized glucose oxidase enzyme in the aerated systems.[10,11] Combinations of pullulanase with amyloglucosidase and glucose oxidase have been attempted to utilize the glucose released in the amylase reaction for bleaching of cotton fabrics.[12] In this present work, a one-step desizing and bleaching of the gray cotton fabrics using α amylase—glucose oxidase combination has been explored and the factors that control the efficiency of the process have also been elucidated.

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EXPERIMENTAL

Gray cotton fabric

Woven cotton fabrics manufactured using warp yarns sized with starch (∼ 94%), mutton tallow (∼ 2.5%), glycerine (∼ 2.5%), and antimildew agent (∼ 1.0%) were used in all the experiments, without any pretreatment. Ends per centimetre (34/cm) and picks per centimetre (25/cm) of the fabrics were measured as per the ASTM D 3775 - 96, linear density of the warp (30 tex) and weft (35 tex) yarns were calculated as per the ASTM D 1059-2001 and areal density of the fabric (220 g/m^2) was measured as per the ASTM D 3776-96.

Bacterial culture for amylase production

Bacillus amyloliquefaciens (MTCC 610), for the production of amylase, was obtained from the Institute of

Microbial Technology, Chandigarh, India. Original lyophilized culture was used for the preparation of mother culture, which was subsequently used for fermentation with one loop-full of organisms. Nutrient agar plate method was used for subculturing the microorganism, required for submerged fermentation method.

Amylase production using submerged fermentation

Bacillus amyloliquefaciens cultures were grown in a 100-mL broth containing soluble starch (1.0% w/v) with peptone (0.5% w/v), $MgSO_4.7H_2O$ (0.2% w/v), $(NH_4)_2HPO_4$ (0.2% w/v), $CaCl_2.2H_2O$ (0.05% w/v), K_2HPO_4 (1.4% w/v), KH_2PO_4 (0.6% w/v), taken in the 500-mL Erlenmeyer flask. The cultures were incubated in the incubator-shaker at a speed of 120 rpm and at a temperature of 37°C for 48 h.[13] Bacterial growth in the inoculums was assessed using absorbance value (optical density) of the sample drawn from the culture medium, with UV-Visible spectrophotometer (Perkin–Elmer, Lambda 35) at regular intervals.

Purification of enzyme

Samples drawn from the fermentation broth were centrifuged at 7000 × *g* for 10 min at 4°C, supernatant obtained from the centrifuge were collected and used for the measurement of protein content using bovine serum albumin solutions as the standard,[14] enzyme activity measurement, and characterization studies for pH and temperature activity.

Measurement of amylase activity using reducing sugar method

Starch solution was prepared by heating a suspension of starch in the reaction buffer until a homogeneous, viscous solution was obtained. Half milliliter of purified amylase enzyme, diluted in acetate buffer of pH 5.5 and 0.5 mL of 1% starch were mixed well and incubated at 55°C for 15 min. To this, 0.5 mL of 3, 5 dinitrosalicylic acid (DNSA) was added and boiled for 30 min before adding 1 mL of sodium potassium tartarate and cooling the contents. Absorbance at the wavelength (λ_{max}) of 540 nm was measured, subtracted from that of enzyme blank, and translated into glucose production using the calibration curve.[15] One unit of amylase was defined as the amount of enzyme that liberates one mole of reducing sugars, measured as glucose per minute (Units/mL/min) under the conditions of assay.

Optimum temperature of amylase was determined by measuring the amylolytic activity using DNSA activity measurement method at temperatures between 30 to 70°C with an incubation period of 30 min. Similarly, optimum pH of amylase was also measured by incubating the enzymes using acetate buffer (pH 3.0–5.9) and phosphate buffer (pH 6.0–7.0).

Desizing with amylase

A piece of gray cloth (20 cm × 20 cm) was used for desizing using the amylase with a concentration of 6840 Units (8 mL/L) at a temperature of 50°C for 50 min at a pH value of 5.5, using the material-to-liquor ratio of 1 : 30. After completion of the process, the samples were washed thoroughly and taken for further assessment. Duration of desizing and concentration of amylases were selected based on the initial experiments carried out using gray fabric samples at various levels.

Weight loss after desizing was calculated as the ratio between the difference in weights, before and after the treatment, to the original weight of the sample. Before weighing, samples were allowed to reach the equilibrium under the standard test conditions with a relative humidity of 65% ± 2%, at a temperature of 25 ± 2°C.

Extractions using solvent mixtures of analytical grade methyl alcohol and benzene (150 mL/g of sample at 2 : 3 ratio) were carried out on the samples obtained from enzyme treatment and control sample with soxhlet extraction apparatus, as specified in the ASTM D2257 - 2004, at the rate of six extractions per hour for a total duration of two hours. The quantity of residual impurities (%) was calculated as the ratio of difference in the weights of the specimen before and after extraction to the original weight of the sample.

Characterization of glucose oxidase activities

Glucose oxidase of *Aspergillus niger* (Himedia, India) was used in all the experiments after characterizing for different temperature and pH values. One unit of glucose oxidase was defined as that amount which produced one micromole of hydrogen peroxide per minute. Effect of temperature on glucose oxidase enzyme was determined by measuring the release of hydrogen peroxide by conversion of glucose (10 g/L) at the temperatures between 37 and 60°C, with incubation periods ranging from 30 to 90 min, at different pH values from 4 to 8. Glucose oxidase concentration was maintained at 40 Units/mL (0.04 mg/mL) of glucose solution and hydrogen peroxide released in the reaction was measured by titration with permanganometric method.[16] The reactions were carried out in a shaker bath, at a speed of 150 revolutions per minute and the mechanical agitations were enhanced by the addition of 10 glass balls, each weighing 1 g. Oxygen

from a pressurized cylinder was supplied into the reaction bath to extent of 5 L/min.

One-step desizing-bleaching process

On completion of desizing duration, the reaction bath was cooled down to 37°C, glucose oxidase enzyme was added (quantity as specified in the discussions) to the same bath without draining. Standing bath was maintained for a duration of 90 min for the conversion of glucose into hydrogen peroxide. Bleaching was carried out, using alkaline activation method,[17] at a temperature of 90°C for 60 min at a pH of 11. After bleaching, the samples were washed using hot and cold water thoroughly.

Surface morphology

Surface morphology of fabric samples were assessed using scanning electron microscope (SEM, Jeol 6390) with a small piece of treated sample and untreated sample at 1000× magnification for assessing surface morphology of the samples and detection of fiber degradation in the process.

Iodine test

A piece of fabric sample was placed in a beaker containing 50 mL of distilled water, boiled for 30 min in a water bath, contents were cooled sufficiently and extracts were transferred to a test tube. Few drops of iodine solution (0.01*N*) were added to the test tube and change in the color was observed, visually.[18]

Whiteness measurement

Whiteness of the bleached fabrics (represented in CIE units) was determined with the reflectance value using i5 Macbeth visible spectrophotometer and the standard illuminant D_{65}. Whiteness values were measured in four different places in the samples and the average values were taken for results and discussion.

Drop absorbency

Drop absorbency of the samples was calculated as per AATCC Test Method 79 - 2000. A drop of water was allowed to fall from a fixed height of 1.0 cm on to the taut surface of test specimen and time required for specular reflection of the water drop to disappear was recorded using a stop watch. Average of 15 tests was taken for the report and further discussions.

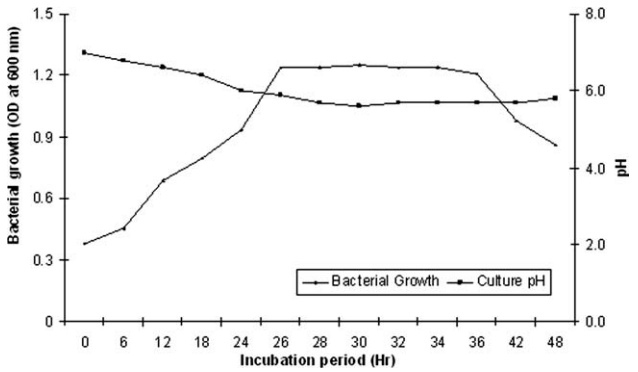

Figure 1 Bacterial growth and pH of starch medium.

FTIR spectrum

To ascertain the functional groups present in the samples, FT-IR spectra were obtained using Shimadzu FT-IR 8400S Spectrophotometer. The control, desized, and desized-bleached fabric samples were mounted on the sample holder and directly taken for the testing. Twenty scans were carried out for every specimen to reduce the influence of the noises and the spectra were obtained between the wave numbers 4000–400 cm^{-1}.

RESULTS AND DISCUSSIONS

Production of amylase and desizing bacterial growth, amylase production, and characterization

Growth pattern of *B. amyloliquefaciens* in the culture, adhered to the typical growth curve,[13] exhibiting growth, stationary and decline phase in the cultures (Fig. 1). Tests conducted in amylase production during early hours of incubation showed sharp changes in the growth and, because of the same reason, time interval for the assessment of bacterial growth was reduced to 2 h after 24 h of postinoculation, measured by the optical density at 600 nm.[19] Maximum growth of bacteria was observed between 28 and 30 h of postinoculation, followed by stationary phase that sustained until 34 to 36 h. Hydrolysis of starch in the medium and formation of glucose led to reduction in the initial pH 7 of the medium to 5.7.

Enzyme secretion (protein content) in the bacterial cultures followed similar trend as that of bacterial growth. Peak enzyme production in terms of protein concentration was about 440 mg/dL, between 28 and 30 h of postinoculum, which translated to the enzyme activity of 871 U/mL/min. Hydrolysis of the amylases, increased apparently in a linear fashion from pH 3.0 and maximum pH activity (1106 U/mL/min) was observed in between the pH 5.8–5.9, followed by a decline phase due to inactivation of amylases at higher pH levels [Fig. 2(a)]. In the case of thermal characterization, maximum

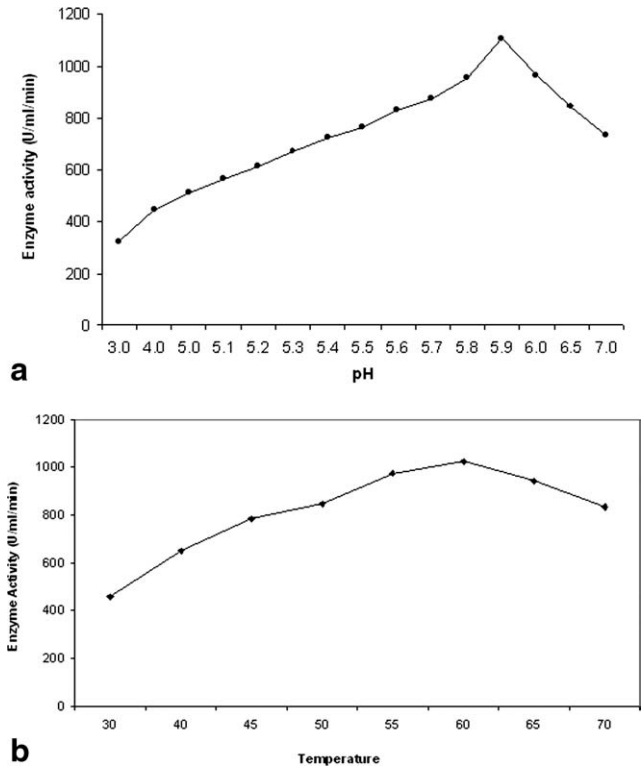

Figure 2 (a) Characterization of amylases for optimum pH 2. (b) Characterization of amylases for optimum temperature.

activity (1026 U/mL/min) was observed, at the temperatures between 55 and 60°C, followed by a decline in the activities further, obviously due to thermal inactivation [Fig. 2(b)].

Desizing of cotton fabrics using amylase

Desizing of gray cotton fabrics using amylase obtained from *Bacillus amyloliquefaciens* resulted in the weight loss of 5.2%, amounting to 87% size removal from the fabric samples. The extractions obtained from the desized fabric samples did not cause any discoloration of iodine solution, while dark blue color was obtained in the case of the untreated samples. However, the residual impurities from the desized fabrics, assessed using solvent extraction, were found to the extent of 3.53% on account of other natural impurities present in the samples.[20]

Bleaching of cotton using glucose oxidase

Maximum release of hydrogen peroxide by glucose oxidase was observed at a pH of 5.0. Magnitude of hydrogen peroxide released in the reaction differed with reference to temperature of incubation and, the maximum release was observed at a temperature of 37°C.

Bleaching of cotton fabrics carried out without the oxygen supply or mechanical agitation did not increase the whiteness values of the fabrics (CIE whiteness index of 29.06; Fig. 3); however, reduction in the whiteness of the bleached samples was observed due to discolouration of reaction bath to dark brown color, which in turn caused fast tinting effect on the fabric samples. Such discolouration often occurs, in the case of D-glucose, at elevated temperatures in alkaline conditions, a characteristic reaction of D-glucose.[21] This obviously necessitates higher concentration of glucose oxidase or longer incubation time for increasing the conversion of reducing sugar into hydrogen peroxide. In spite of similar concentrations of glucose and process conditions used in glucose oxidase bleaching process, phenomenon of discolouration has not been reported in the literature.[10,12,22] When gaseous oxygen was supplied from a pressurized cylinder to the extent of five liters per minute, whiteness values showed marginal improvement only.

Combined desizing and bleaching

Reducing sugar released in the desizing process was utilized for conversion into hydrogen peroxide, without further addition of glucose, using glucose oxidase enzyme. Glucose oxidase 40 Units/mL was added to the cooled desize bath and incubated for the conversion of glucose into hydrogen peroxide, with the introduction of gaseous oxygen into the reaction bath. Though hydrogen peroxide, at acidic pH, releases hydroxyl radicals that result in tendering of cellulose, such reactions require high temperature conditions,[23] which did not prevail in the present study. Hydrogen peroxide released in the process was activated using sodium carbonate and bleaching was carried out for 60 min at 90°C. Higher concentration of glucose oxidase (60 Units/mL) resulted in significantly higher whiteness index up to 52.30 (CIE units), with the same level of glucose in the bath, which further increased to 73.6 with the glucose oxidase concentration of 80 Units/mL, much

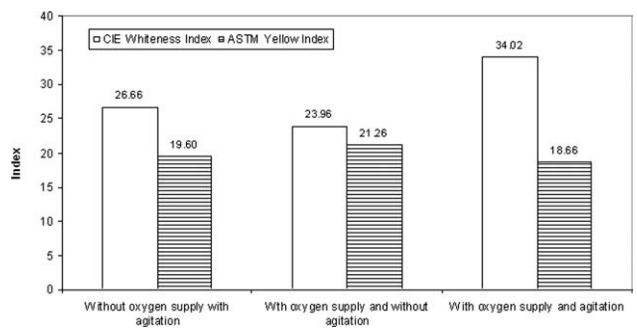

Figure 3 Effect of mechanical agitations and oxygen supply on bleaching.

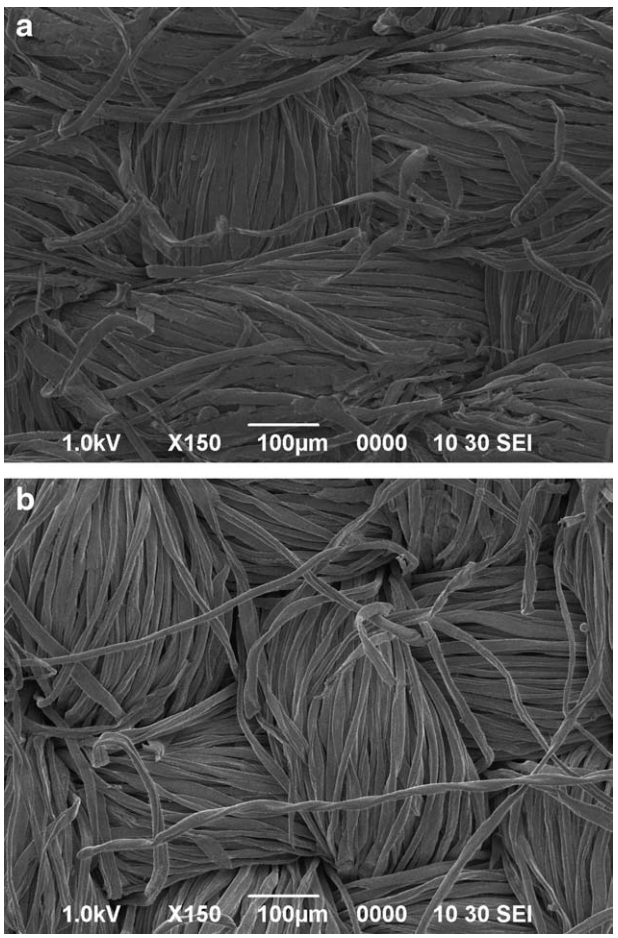

Figure 4 SEM Images of (a) untreated and (b) one step desize-bleach samples.

closer to the CIE whiteness values (73.0–74.0) obtained in the commercial bleaching processes using hydrogen peroxide.[10]

Surface morphology

Surface scans of the bleached samples using glucose oxidase appeared very much clear, free from size ingredients, observed in the untreated sample [Fig. 4(a)] that made the fibers to stick to each other in many places and surface degradations were not visible in the samples obtained from one-step desizing-bleaching process [Fig. 4 (b)] that are often observed in the commercial treatment. Longitudinal grooves of the fibers were visible in the enzyme bleached samples, showing clear surface with the natural twists present in the individual fibers, after combined desizing-bleaching process.

Drop absorbency

Presence of hydrophobic impurities on the fiber surface, dried film of starch and other hydrophobic components (tallow) in the sized yarns, contribute to lower absorbency of the gray fabrics. Treatment with mesophile amylases, carried out at lower temperatures, often results in the incomplete removal of starch and other size ingredients attached to it, resulting in lower absorbency levels.[24] The drop absorbency values of desized fabrics showed the lowest absorbency value time of 24 s, however, when the bleaching treatment is combined with desizing, the residual constituents are expected to be removed and enhance the absorbency of the fabrics. Interestingly, in the case of samples obtained from the one-step desizing-bleaching process, the lowest absorbency value was observed at 2 s and the highest value at 49 s, showing strong influence of the residual impurities (2.13%) and effectiveness of the oxidation treatment in the combined process.

FTIR results

In the case of desized samples, FTIR peaks at 700–400 cm^{-1}, of *N*-H wagging attributable to protein residues were visible, similar to 2200–2000 cm^{-1} (coloring matters), 1700–1600 cm^{-1} (pectin substances), 2900–2800 cm^{-1} (alkyl ester chains of cotton wax and tallow) and 2400–2200 cm^{-1} (*N*-H stretch of secondary amides). Strong peaks (3850–3600 cm^{-1})

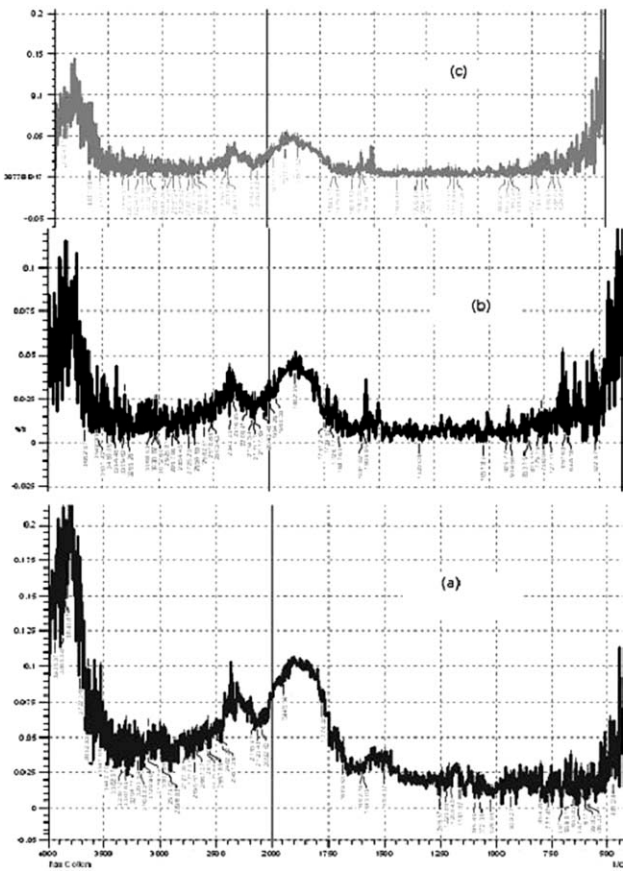

Figure 5 FTIR spectrograph of (a) untreated (b) desized and (c) one-step process sample.

attributable to the O—H stretch in the primary and secondary hydroxyl groups of cellulose became more predominant in all the samples as seen in the untreated samples [Fig. 5(a,b)]. The samples obtained from the one-step process showed less intensive, minor peaks due to removal of many of the impurities from the gray cotton fabrics [Fig. 5(c)]. Peaks ascribed to flavnoids (1660–1620 cm^{-1}) and coloring nitrogen compounds (2777 cm^{-1}) were not observed in the samples obtained from one-step process, demonstrating the bleaching action of hydrogen peroxide and removal of colored compounds.[25] Peaks in the 3690–3600 cm^{-1}, due to O—H stretch in the primary and secondary hydroxyl groups became more predominant, due to reduction of the impurities that were originally masking the cellulose, in the bleached cotton samples. Peaks at 1000–940 cm^{-1} became prominent, associated with the C—O—H out-of-plane bending, due to —CH_2OH, C_1—C_4 linkage in the β turn of glucose residues in cellulose.

CONCLUSIONS

Drop absorbency of the amylase desized fabrics showed lower values, which increased significantly in the samples obtained from one-step desizing-bleaching process using amylase–glucose oxidase enzymes. However, the presence of residual glucose in the bleaching process has the potential to reduce whiteness of the fabrics under alkaline pH at high temperatures during alkaline activation in bleaching reaction. This obviously necessitates high concentrations of glucose oxidase in the bleaching process, besides external supply of oxygen and mechanical agitations. Whiteness value of the samples treated using combined desizing–bleaching was very much closer to the values achievable in the commercial treatments. The process also resulted in the complete removal size ingredients with lower levels of extractable impurities in the samples. The one-step process can be commercially exploited in place of the harsh chemical treatment, which deteriorates the fibers and fabrics in the process.

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